CLAIMS

1. A method of multiple microorganism detection which is a method for detecting two or more microorganisms having different properties in foods, with high sensitivity comparable or even superior to official methods, by amplifying a plurality of target genes with a single PCR reaction tube and analyzing the same, comprising the following steps:
(A) a step for extracting DNA of the target microorganisms to be detected, by treating at least with a lytic enzyme and /or bacteriocin having lytic activity, a surfactant and a protein denaturant; and
(B) a step for performing Multiplex PCR by mixing a primer specific to the target microorganisms to be detected.

2. The method of multiple microorganism detection according to claim 1, wherein a step to culture microorganisms under a culture condition where 1 CFU/100 g microorganisms become 10^3 CFU/ml or more after 24 h of culture, is included prior to the step of extracting DNA of the target microorganisms to be detected.

3. The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise Listeria monocytogenes.

4. The method of multiple microorganism detection

according to claim 3, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 5 and 6.

5. The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise pathogenic Escherichia coli O157.

6. The method of multiple microorganism detection according to claim 5, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 1 and 2, or SEQ ID Nos: 7 and 8.

7. The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise Salmonella spp.

8. The method of multiple microorganism detection according to claim 7, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 3 and 4, or SEQ ID Nos: 9 and 10.

9. The method of multiple microorganism detection according to any one of claims 1 to 8, wherein the microorganisms are cultured in a culture condition where the pH after culture becomes 5.1 or more.

10. The method of multiple microorganism detection according to any one of claims 1 to 9, wherein the microorganisms are cultured in a medium with glucose concentration of 0.15% or less, and/or in a medium with concentration of phosphate-buffer solution of 50 mM or more or in a medium with a buffer ability similar as that with concentration of phosphate-buffer solution of 50 mM or more.

11. The method of multiple microorganism detection according to any one of claims 1 to 10, wherein the extraction is performed after treating with a lytic enzyme and/or bacteriosin having a lytic activity, further treating with a surfactant and a protein denaturant, removing insoluble fractions by centrifugation, and by depositing DNA by alcohol precipitation.

12. The method of multiple microorganism detection according to any one of claims 1 to 11, wherein the lytic enzyme is Achromopeptidase and/or lysozyme.

13. The method of multiple microorganism detection according to any one of claims 1 to 12, wherein bacteriosin having lytic activity is Enterolysine.

14. The method of multiple microorganism detection according to any one of claims 1 to 13, wherein the surfactant is ethyleneoxide condensate of sorbitan monolaurate.

15. The method of multiple microorganism detection according to any one of claims 1 to 14, wherein the protein denaturant is Guanidine isothiocyanate.

16. The method of multiple microorganism detection according to any one of claims 1 to 15, wherein Multiplex PCR is performed by combining DNA consisting of base sequences shown by SEQ ID NOs: 1 to 6 at a total concentration of 750 nM or less as a primer.

17. The method of multiple microorganism detection according to any one of claims 1 to 15, wherein Multiplex PCR is performed by combining DNA consisting of base sequences shown by SEQ ID NOs: 5 to 10 at a total concentration of 750 nM or less as a primer.

18. The method of multiple microorganism detection according to any one of claims 1 to 17, wherein the food is edible meat or processed meat product.